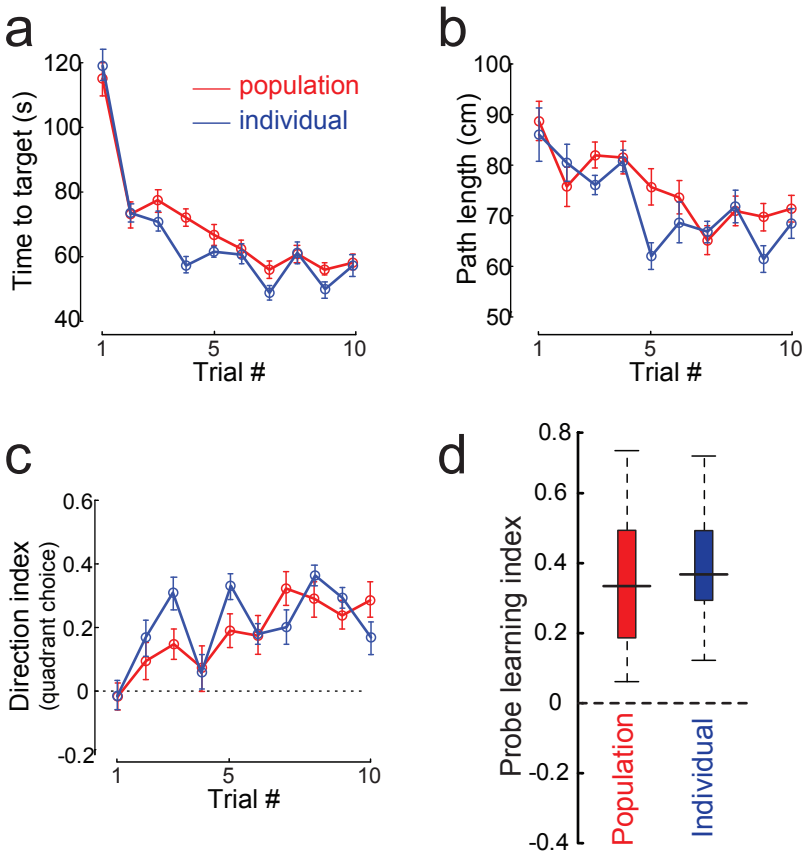
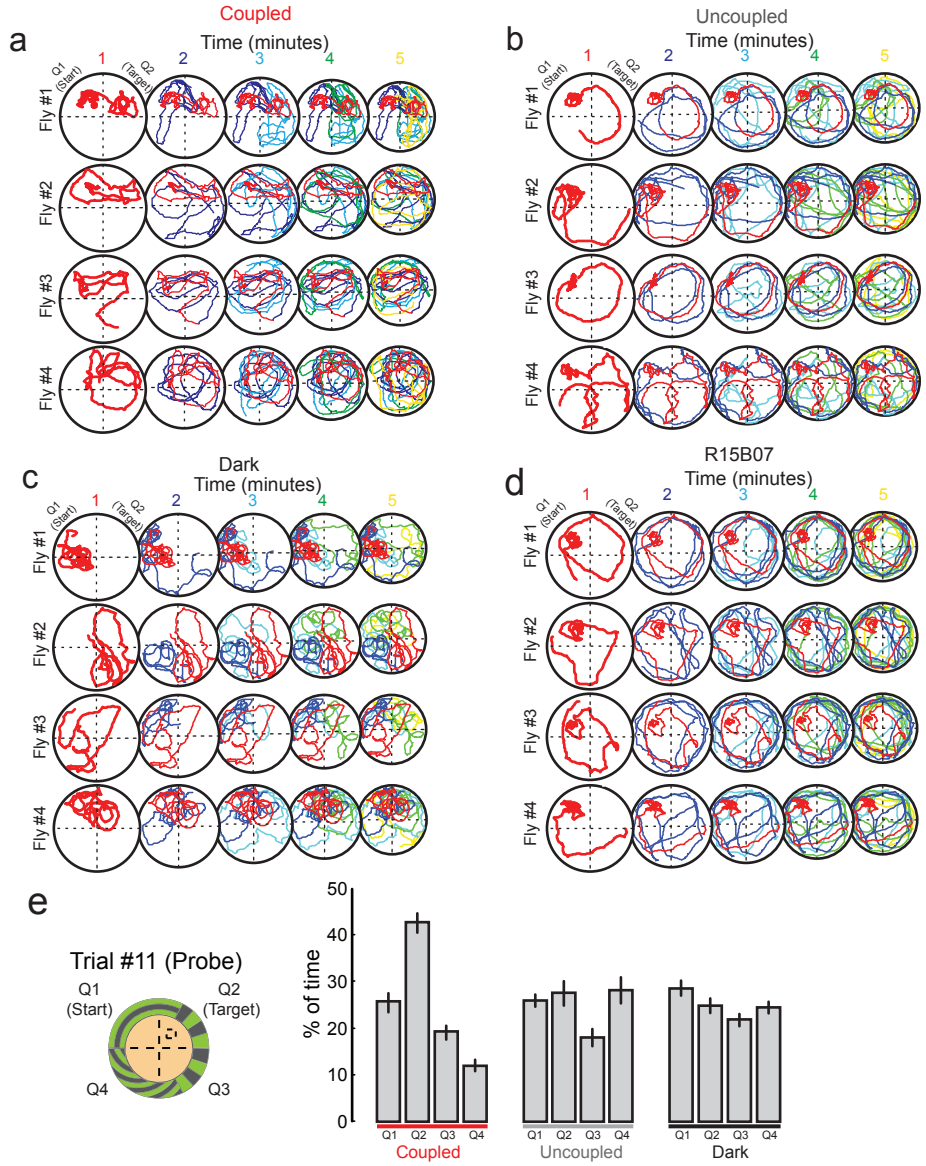
Supplementary 1



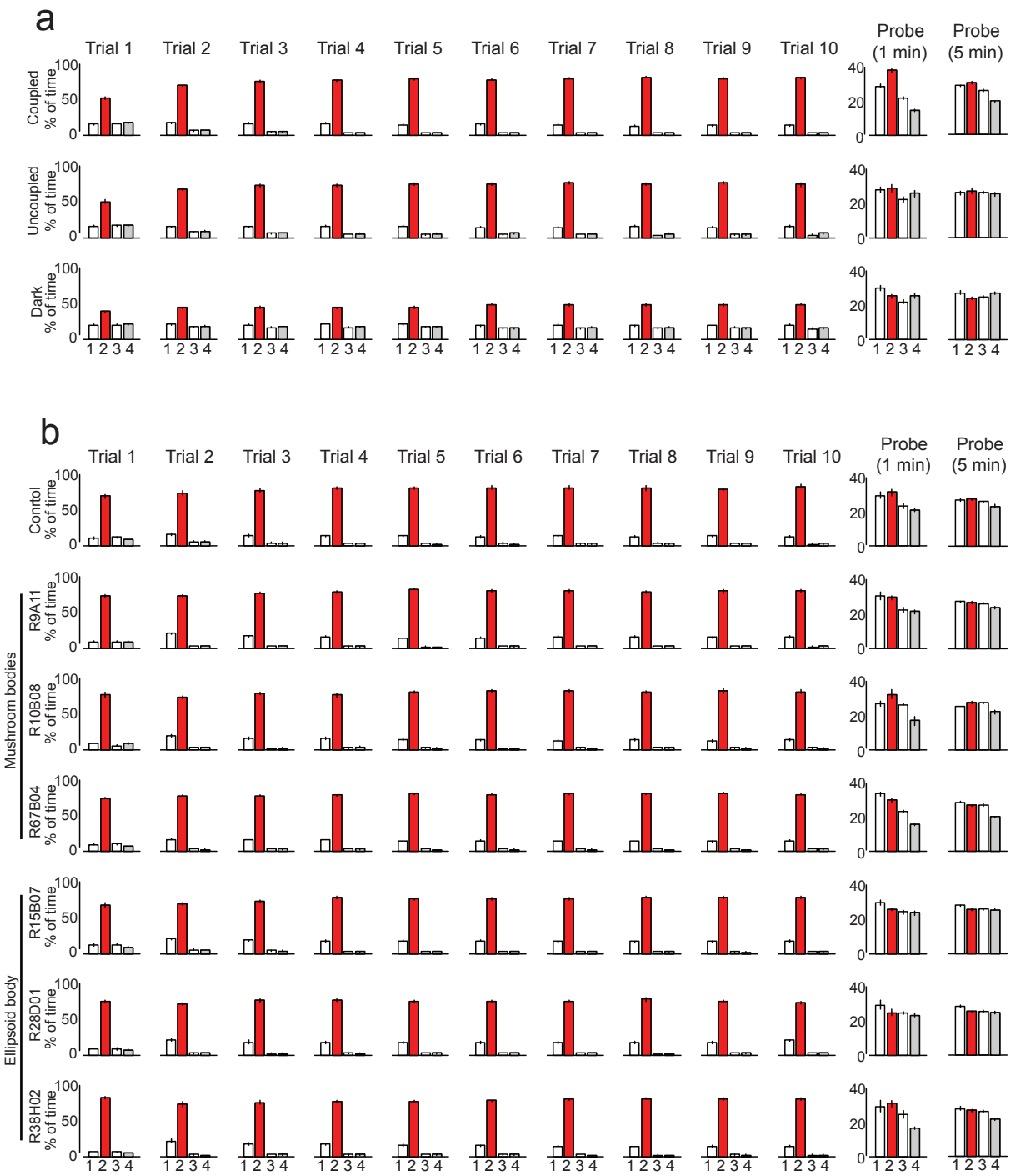
Supplementary Figure 1: Flies tested individually behave comparably to flies tested as a population. (**a-d**) To assess the possibility of social interactions influencing place learning behavior, flies were tested either as a population (red, 25 experiment, 15 flies per experiment), or individually (blue, 30 flies). For comparison, the results from individual flies were randomly sampled to generate 25 groups composed of 15 flies each. (**a**) There is no noticeable difference in the time to target, (**b**) path length, (**c**) direction index, or (**d**) probe learning index when comparing flies tested individually to flies tested as a population. (**a-c**) Values represent mean ± SEM or (**d**) as described in Fig. 3.

Supplementary 2



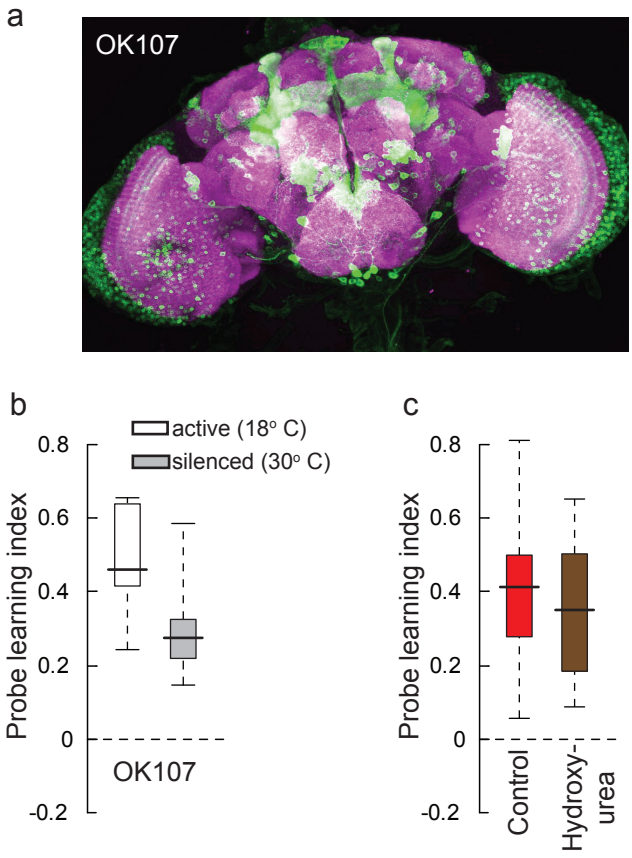
Supplementary Figure 2: Flies trained with a coupled visual panorama preferentially search in the target quadrant during the probe trial. (**a-c**) Trajectories of 4 representative wildtype flies during a probe trial after training with a (**a**) coupled, (**b**) uncoupled, or (**c**) dark visual panorama. Trajectories are color coded by minute (in order red, dark blue, light blue, green, yellow). Q1 (start) is the location of the cool tile in the preceding trial and Q2 (target) is the location that the cool spot would be (as indicated by the visual panorama). Note that in addition to preferentially searching in Q2 (the target quadrant), wildtype flies trained with a coupled visual panorama return repeatedly to Q1 (the location of the cool tile in the previous trial). (**d**) Trajectories plotted as in (a-c) for 4 representative eb silenced flies (line R15B07) trained with a coupled visual panorama. (**e**) Wildtype flies trained with a coupled visual panorama (red, n=33) preferentially search Q2, the quadrant where they have been trained to locate the cool tile. When trained with an uncoupled panorama (gray, n=21) or in the dark (black, n=23), flies display no spatial search bias and explore the arena uniformly. Percentage of time is calculated during the first 60 seconds after flies leave Q1.

Supplementary 3



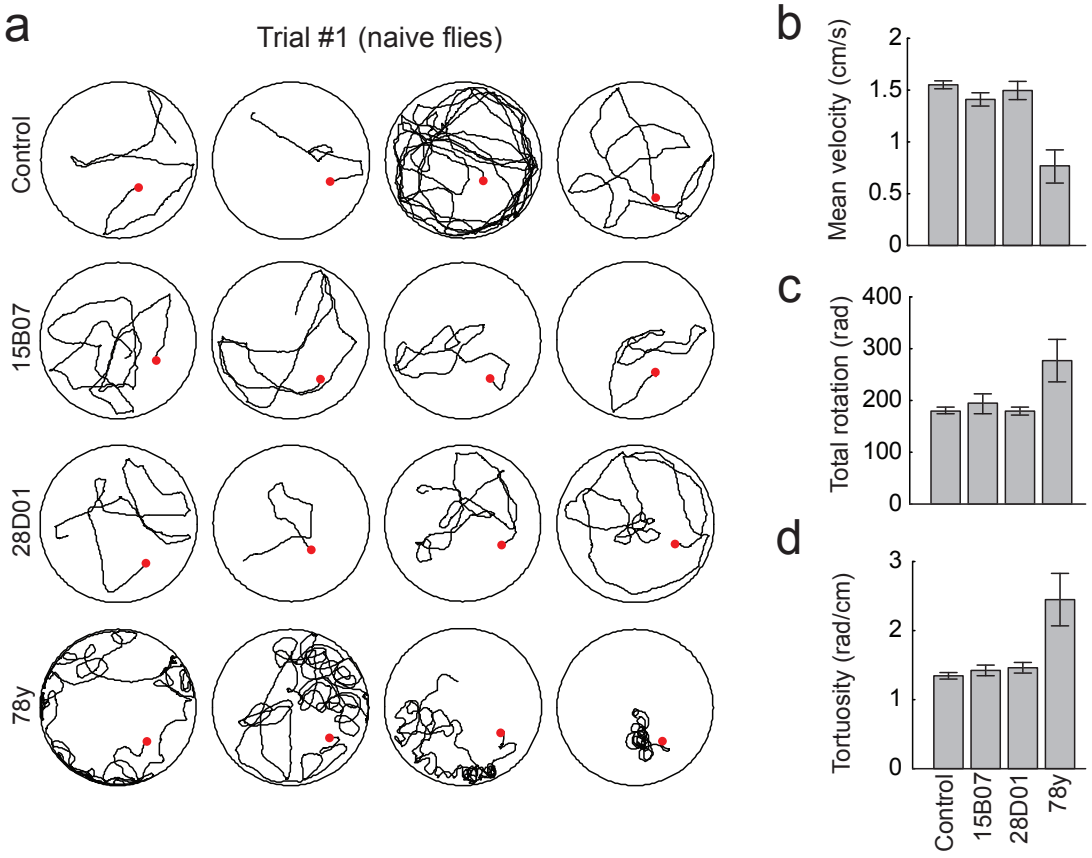
Supplementary Figure 3: Flies show no spatial search bias in the probe trial when trained in the dark, when trained with an uncoupled visual panorama, or when eb circuits are silenced (a,b) The percentage of time flies spend in each of the 4 quadrants is illustrated for the training trials (Trial1-10), the first 60 seconds of the probe trial, and the full 5 minutes of the probe trial. **(a)** Results for wildtype flies trained in the coupled, uncoupled, or dark condition, **(b)** or GAL4 driver lines following induction of Kir2.1 trained with a coupled visual panorama are shown. Quadrant numbering follows the convention in Fig. 3.

Supplementary 4



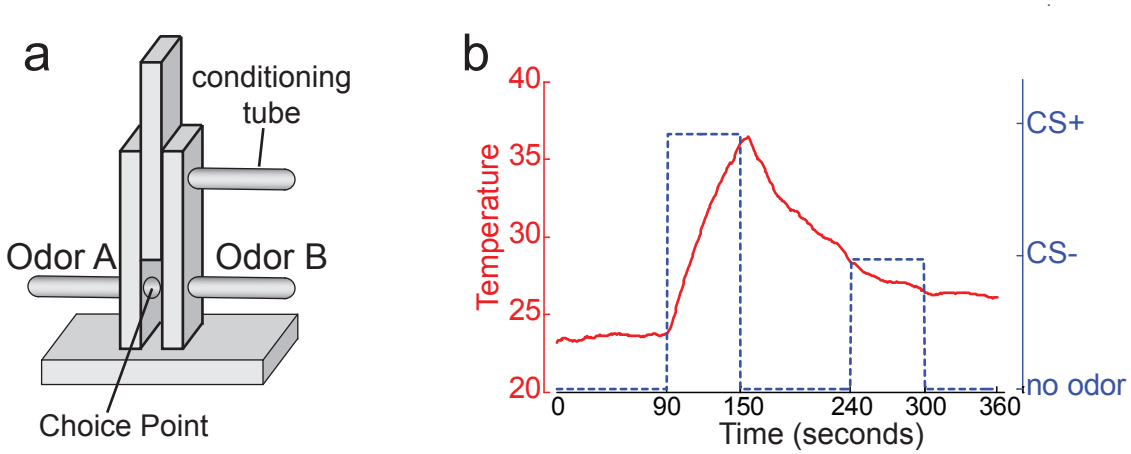
Supplementary Figure 4: Mushroom bodies are not required for visual place learning. (a, b) The pan-mushroom body GAL4 driver line OK107 was used to conditionally silence neurons in the adult brain. **(a)** Shown is the expression pattern for the OK107 driver following expression of a GFP reporter. (**b)** White boxes denote spatial learning performance of the OK107 line prior to Kir2.1 induction; grey box indicates the performance of sibling flies following Kir2.1 expression. No significant place learning impairment is seen after Kir2.1 induction (one-tailed t-test, $p<0.05$, n=10 experiments, 150 flies for each condition). **(c)** Likewise, ablation of the mushroom bodies using hydroxyurea has no significant effect on place learning abilities when compared to sham treated siblings "Control" (one-tailed t-test, $p<0.05$, n=5 experiments, 75 flies for each condition). Box plots are as described in Fig3. The brain image in (**a**) was provided by Julie Simpson and Phuong Chung.

Supplementary 5



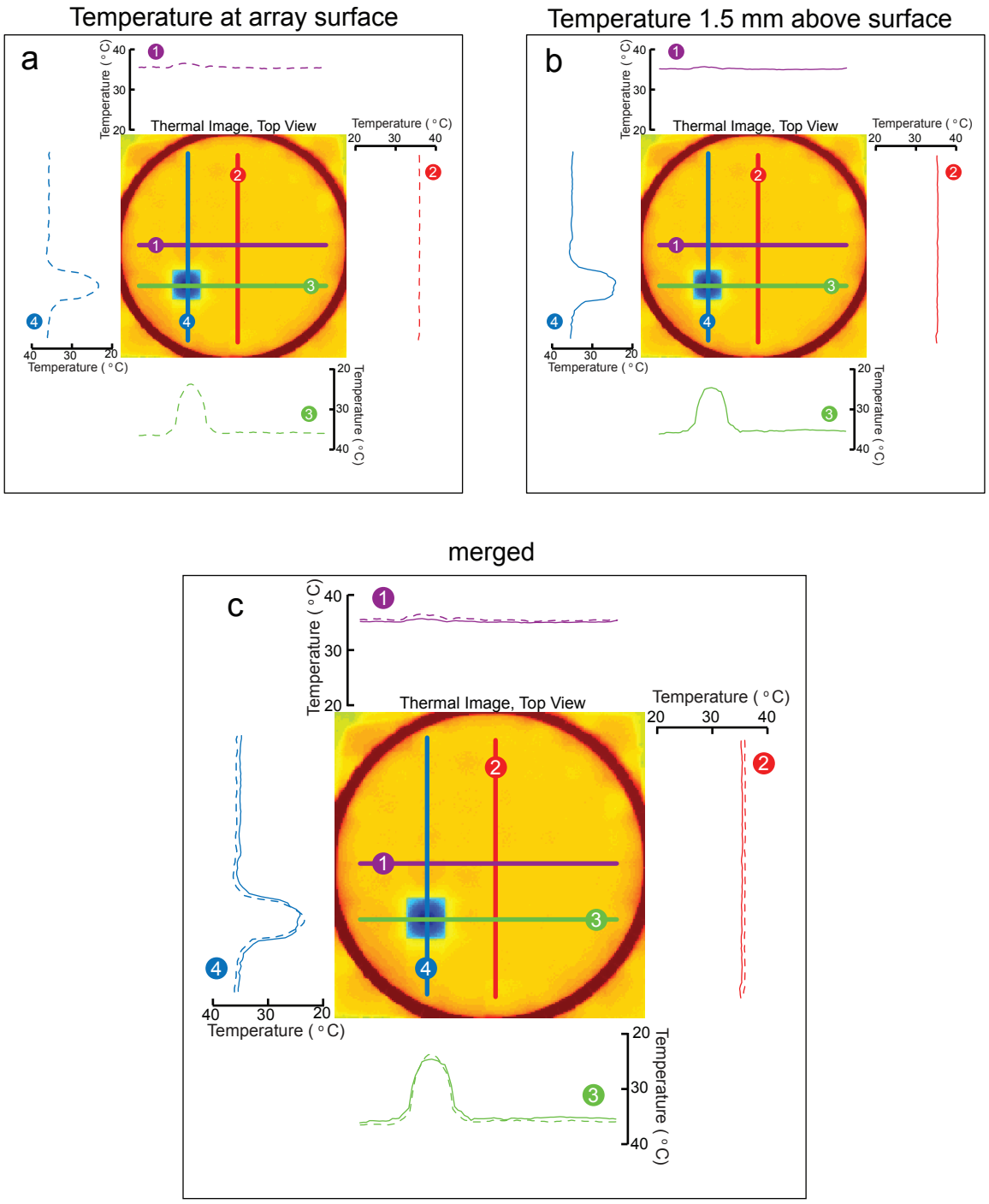
Supplementary Figure 5: Place learning impairments in lines R15B07 and R28D01 are not caused by locomotor abnormalities. (a) Trajectories from 4 representative naïve flies in trial #1 are shown for control flies, lines R15B07, R28D01, and 78y, all after induction of Kir2.1. The red dot denotes the point at which flies locate the cool spot. Note no apparent abnormalities in the trajectories of R15B07 and R28D01 when compared to control trajectories. For comparison, refer to line 78y which has documented locomotor impairment[31]. **(b-d)** Likewise, lines R15B07 and R28D01 show no abnormalities in **(b)** walking velocity, **(c)** cumulative rotation, or **(d)** tortuosity (the ratio of the accumulated rotation over the accumulated distance traveled) of their paths when compared to control flies. Abnormalities in motor control are easily observable in line 78y using all of these metrics.

Supplementary 6



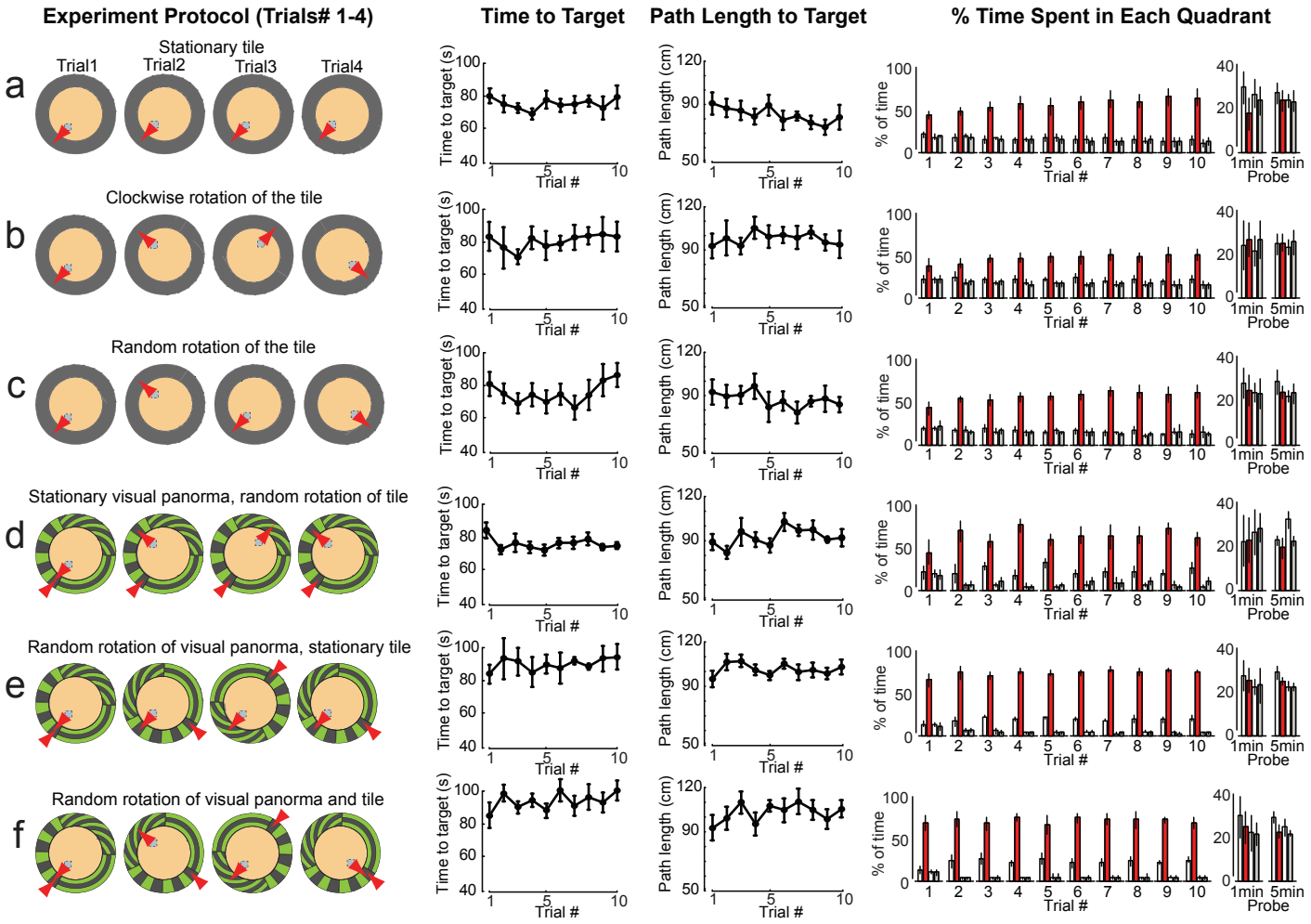
Supplementary Figure 6: A novel olfactory conditioning paradigm using heat as the US. (**a, b**) Flies were conditioned to avoid one of two odors using an elevated T maze and heat (rather than electric shock) as the unconditioned stimulus (See methods). (**a**) Flies were initially place in the conditioning tube and exposed to one of two odors, 4-Methylcyclohexanol (Odor A) or 3-octanol (Odor B), paired with heat. Following exposure to the first odor, flies were presented with the alternate odor in the absence of heat. After this training, flies were lowered into the choice point and allowed to distribute between a tube containing Odor A or Odor B. (**b**) Conditioning regime: flies are trained with the CS+ and CS- stimulus for a total of 6 min prior to testing; dashed blue lines show the time window of odor delivery (CS+ odor at 90 sec and CS- odor at 240 sec), red trace indicates the temperature in the conditioning tube during the training.

Supplementary 7



Supplementary Figure 7: The temperature-controlled arena floor exhibits no thermal gradients that flies could follow towards the cool tile. Temperature readings (± 0.1 degree) were made across the TEM array at locations indicated by lines 1 to 4 superimposed on a thermal image of the array. (**a**) Temperature readings at the array surface (dashed line, data from thermal imaging camera) or (**b**) a thermocouple 1.5 mm above the array surface (solid line). (**c**) Temperature readings from panels **a** and **b** plotted on the same axis; note a slight temperature difference in the vertical direction. No gradients are detected significantly beyond the boundaries of the test cool tile.

Supplementary 8



Supplementary Figure 8: Idiothetic cues are not sufficient to guide flies to the cool spot. If (non-visual) path integration were used by flies while navigating the large arena, we expect that flies might improve in their ability to find the cool tile when trained in the dark with (**a**) the target consistently in the same physical location, or (**b**) consistently rotated in the same direction (e.g. relocated 90º clockwise). Flies were trained for 10 trials, with trials 1-4 shown in the illustrations (left panels). Also shown are time to target, path length to target, and percentage of time flies spend in each of the 4 quadrants for the training trials and the probe trial (red = target quadrant, quadrants 1,3,and 4 are indicated by open or shaded bars as in Supplementary Fig. 3). (**c**) The experiment was also performed with the tile randomly relocated in each trial. No notable spatial learning is observed with flies trained using any of these experimental paradigms (compare to Fig. 1 and Fig. 2). (**d-f**) In a parallel set of experiments, the visual surround was active, but was uncoupled from the location of the cool tile: (**d**) The visual surround was held constant but the cool tile was moved randomly, (**e**) visual surround was moved randomly but the tile was held constant, (**f**) visual surround and cool tile were both moved randomly. For all conditions, n = 8 experiments; values are mean ± SEM.

Supplementary Movie 1: Place learning in the thermal-visual arena. Left: movie (played at 25x recorded rate) shows all flies during training trials 1-10 in a representative coupled experiment. The movie highlights the position of each fly, with its center (dots) and trajectories (lines) in one of 6 colors; the lengths of the tracks denote a 4 s window. The yellow box indicates the borders of the lone cooled tile. **Top right:** Diagram illustrating the displayed visual panorama (gray and green) and the location of the cool spot (blue square). Note that even as the absolute position of the cool spot changes between trials its location relative to the visual panorama remains constant (coupled condition). **Bottom right:** Median time to reach the cool tile (red dot) is shown. For each trial, data is plotted after >90% of the flies reach the cool spot. Note the dramatic improvement in the time to reach the safe spot as the flies learn its location (i.e. between trials 1 and 3).

Supplementary Movie 2: Probe trial following training with a coupled visual panorama. Left: movie shows flies (played at 2x recorded rate; the very same flies as in Supplementary Movie 1) in the probe trial (trial #11) following training with a coupled visual panorama. Flies are individually colored red if they first reach the "imaginary" cool tile (red square, i.e. the location where the visual panorama indicates the cool spot would be, even though no cool tile is present; see text for details), or yellow if they first reach the corresponding tile in the opposite quadrant (yellow square). **Top right**: Diagram indicates the displayed visual panorama (gray and green). Q2 (Target) is the quadrant where the flies have been trained to locate the cool tile, and the dotted red line indicates the position of the "imaginary" cool tile. Q4 is the quadrant opposite the target; flies showing no learned place preference should visit this quadrant with equal

probability as Q2. **Bottom right:** Bar plots for individual flies show the % time each fly spends searching in Q2, Q3, or Q4 after leaving the starting quadrant (Q1).

Supplementary Movie 3: Probe trial following training with an uncoupled visual panorama. Left: movie (played at 2x recorded rate) shows all flies in the probe trial (trial #11) following training with an uncoupled visual panorama. Flies are individually colored red if they first reach the "imaginary" target tile (red square), or yellow if they first reach the corresponding tile in the opposite quadrant (yellow square). Because flies trained with the uncoupled panorama cannot use the visual display to locate the target tile, they should show no guided navigation to Q2 versus Q4, and therefore visit both quadrants with equal probability (see supplementary movie 2). **Top right**: Diagram indicates the displayed visual panorama (gray and green). **Bottom right:** Bar plots for individual flies show the % time each fly spends searching in Q2, Q3, or Q4 after leaving the starting quadrant (Q1).